

but evidently he is not applying those rules as they were applied (we believe correctly) at the EPO during International Preliminary Examination of the Parent International Application. The pending claims are equivalent to the amended claims of the Parent International Application as annexed to the International Preliminary Examination Report. A copy of that report was provided when requesting US national phasing of the Parent International Application. As would be anticipated for correct application of PCT Rules 13.1 and 13.2 at the EPO, no lack of unity was raised in the International Preliminary Examination Report (IPER) by the EPO Examiner. Indeed, all the amended claims filed in response to the Written Opinion at the EPO were held in the IPER to be linked by a special technical feature which provides both novelty and inventive step, more particularly use of the peptide represented by SEQ ID No. 1 (the peptide designated ES1 corresponding to the N-terminal 15 amino acid residues of the ESAT-6 protein of *M. tuberculosis*) or an analogue thereof which will bind the same T cell receptors. The same applies for all the claims in the instant application.

Claim 27 directs use of the peptide of SEQ ID No 1 alone or together with one or more further peptides as part of a peptide panel for the purpose of determining infection of a human with a Mycobacterium expressing ESAT-6, especially *M. tuberculosis*. The same applies to independent claim 28.

Independent claim 75 is directed to use of a specified peptide panel consisting of all the peptides corresponding to SEQ ID NOs: 1 to 8 for diagnosing, for example, *M. tuberculosis* infection in humans. This is the same peptide panel as specified for the same purpose in dependent claim 30.

Claim 27 is specifically directed to *in vitro* diagnosis while claim 28 covers determination of *M. tuberculosis* infection *in vivo* in humans by administration of the peptide of SEQ ID No 1 or a peptide panel including that peptide. The same peptide or peptides might be provided *in vivo* for the same purpose by expression from one or more polynucleotides. It is this possibility which is covered by independent method claim 53. Hence, claim 53 is directed to the same invention as claim 27 and claim 28, i.e. use of the peptide SEQ ID No 1 or the peptide of SEQ ID No 1 in a peptide panel to detect a T cell immune response to Mycobacterium infection in humans.

It is evident that the Examiner is applying the restriction requirement criteria normally applied to directly filed US patent applications under the guise of PCT rules. However, even having regard to normal US restriction practice, severance of the Group I, III and VII method claims is objectionable. This is certainly so, if as the Examiner states, the true criterion is a single general inventive concept based on the same special technical feature. A comparison of representative claims of Groups I, III and VII, claims 27, 53 and 75, is informative, to wit:

Group I	Group III	Group VII
27. A method of determining infection in a human patient by, or exposure of a human patient to, a mycobacterium which expresses ESAT-6 which method comprises the steps of:	53. A method of determining infection in a human patient by, or exposure of a human patient to, a mycobacterium which expresses ESAT-6 which method comprises the steps of:	75. A method of diagnosing infection in a human patient by, or exposure of a human patient to, a mycobacterium which expresses ESAT-6 which method comprises the steps of:
(i) <u>contacting</u> a population of T cells from the patient with the peptide represented by SEQ ID NO:1 and, optionally, one or more further peptides selected from the group consisting of the peptides represented by SEQ. ID. NOs: 2 to 11 and	(i) <u>administering</u> one or more polynucleotides expressing in human cells the peptide represented by SEQ ID NO: 1 and, optionally, one or more further peptides selected from the group consisting of the peptides represented by SEQ ID NOs: 2 to 11 and	(i) <u>contacting</u> a population of T cells from the patient with a panel of peptides represented by SEQ. ID. Nos. 1 to 8, wherein said T cells are freshly isolated <i>ex vivo</i> cells from peripheral blood, and
(ii) determining <i>in vitro</i> whether the T cells of said T cell population	(ii) determining whether T cells of the patient recognize said	(ii) determining <i>in vitro</i> whether T cells of said T cell population

recognize said peptide(s).	peptide(s).	show a recognition response to said peptides by determining IFN-* secretion from the T cells.
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Simple inspection demonstrates that representative claims 27, 53 and 75 are all directed to the same type of steps of determining, or diagnosing, infection in a human patient. The first step (i) is the same type of step in all three groups of claims. In claims 27 and 75 it is contacting a population of T cells from the patient with at least two peptides. In claim 27 the peptides are SEQ ID NO:1 and one or more represented by SEQ. ID. NOs. 2 to 11. In claim 75 the peptides are a panel of peptides represented by SEQ. ID. Nos. 1 to 8, so there is overlap between the claims. In claim 53, the same peptides as in claim 27 are "administered", which is simply a broader way of contacting a population of T cells.

* The second step (ii) is also the same type of step in all three representative claims. Claims 27 and 75 call for the determination to be made *in vitro*, while claim 53 is broader in that respect. Claim 75 is narrower in calling for the determination to be by IFN-* secretion from the T cells.

Similar comparisons can be made with the other groups of claims. Please note that the kit composition claims of Groups II and IV refer solely to (i) peptide panels including SEQ ID No 1, or a suitable analogue thereof or (ii) polynucleotides for expression of such a panel. The scope of these claims takes account of the disclosure of the journal paper cited as D2 in the IPER (the Brandt et al. paper in Journal of Immunology, 1996). Not only the kit claims, but also the pharmaceutical composition claims of Groups V and VI share the same key inventive feature specified in claims 27 and 53, i.e. provision (either directly or via a polynucleotide) of the peptide of SEQ ID No 1 (or an analogue thereof which binds the same T cell receptors) to detect a T cell response in humans to *M. tuberculosis* infection.

It is to be noted that section V.4 of the relevant IPER summarizes the case for the significance of the peptide of SEQ ID No 1 as regards inventive step over the

cited prior art and especially D2. There, the Examiner recognized the unity of the inventive step, to wit:

"The present application is based on the surprising finding that the peptide 'ES1' represented by SEQ ID NO:1 and corresponding to amino acids 1-15 of the ESAT-6 protein of *Mycobacterium tuberculosis* is suitable to detect nearly 60% of human TB patients. This finding could not be expected from any of the relevant prior art documents D1, D2, D3 and D7."

The Examiner there went on to detail the reasons for the unexpected nature of the invention and concluded:

"Therefore, novelty and inventive step of the claimed subject-matter is acknowledged."

Elaborating on the foregoing, that SEQ ID No 1 in the claims provides a unifying inventive feature, it was made clear in the reply to the Written Opinion filed in the Parent International Application, that the inventors were the first to identify the importance of a T cell epitope presented by the ES1 fragment of the protein ESAT-6 as regards determining *M. tuberculosis* infection in humans. This represents an important technical contribution to the diagnosis of *M. tuberculosis* infection, which could not be extrapolated from any previous study of T cell responsiveness to ESAT-6 epitopes amongst T cells taken from *M. tuberculosis*-infected animals. Indeed, as expanded below, if anything, animal studies reported before the priority date pointed away from use of peptide ES1, or any other closely-related peptide fragments, for determining human *M. tuberculosis* infection.

As emphasized at lines 24 and 25 of page 1 of the description, peptide ES1 alone was found to detect nearly 60% of TB patients tested. This is comparable with a conventional skin test, but without the problem of false negatives arising from previous BCG vaccination. Thus, while it may be considered preferable to combine peptide ES1 with further ESAT-6 epitope-containing fragments to provide a peptide panel enabling an even higher percentage detection of TB patients, it is evident that peptide ES1 alone enables a clinically useful and indeed advantageous method for detecting *M. tuberculosis* infection in humans compared with conventional TB diagnosis.

In acknowledging inventive step in the relevant IPER and unity of invention, the Examiner for IPE of the Parent International application highlighted that D2 is confined to studies with mice. Although the authors of D2 did find that peptide ES1 contains a T cell epitope recognized by T cells taken from *M. tuberculosis*-infected mice, it is not possible to extrapolate with reasonable expectation of success from that information that the same peptide will prove effective in detecting human TB patients. The Examiner might be usefully referred to the discussion of D2 on page 2 of the description in the paragraph beginning at line 3. As emphasized in that paragraph, "as well as other differences in epitope processing, presentation and recognition, mice have different MHC molecules from humans and thus are expected to recognize different epitopes from humans". As illustration of this point, the information in Table 1 of D2 for peptide p6-20 is worthy of note. That peptide was not identified as an epitope-containing fragment of the ESAT-6 protein by the mice studies of Brandt et al. However, it is identical to the peptide designated ES2 in the subject application (corresponding to SEQ ID No 6), which was found by the inventors to detect 40% of TB patients amongst the TB population tested (see table 1 on page 26). Since the data for peptide ES2 in D2 does not reliably predict efficacy of the same peptide in diagnosing human *M. tuberculosis* infection, the same must apply to the data presented in D2 for peptide ES1.

It is notable that D2 makes no reference to diagnosis whatsoever. The results presented in table 1 of D2 were obtained using splenic or lymph node cells rather than blood-derived T cells. Furthermore, while the authors of D2 used the ELISPOT technique (the same technique as employed by the inventors in this instance), there is no suggestion in D2 to apply that technique for diagnosis in humans with specific ESAT-6 peptides as distinct from merely establishing that ESAT-6 does provide T cells epitopes in mice. D2 only speculates about future vaccines with reference to whole ESAT-6 as a prominent target molecule.

As further support that reference to SEQ ID No 1 in the claims provides a unifying inventive feature, it is also useful to look at the journal paper D7 as cited in the relevant IPER (Elhay et al. Infect. Immun. 1998, 3454-3456). That paper derived from work of the same research group as D2 and was published after D2. D7 provides data showing that a peptide from the C-terminal region of ESAT-6, peptide

p8, is effective in a skin test in detecting T cells in guinea pigs infected with *M. tuberculosis*. However, in the same studies peptide p1 (amino acid residues 1-20 of ESAT-6) gave no significant results. This further illustrates why one cannot extrapolate with reasonable expectation of success from D2 that any peptide from the N-terminus of ESAT-6, including peptide ES1, will be a useful diagnostic tool in humans. Indeed, D7 was published two years after D2 and yet merely gives indication that a good diagnostic reagent for a human skin test reagent is a combination of whole ESAT-6 with another whole protein (see in D7 the paragraph beginning in column 1 on page 3455 and the sentence immediately preceding that paragraph). The fact that Andersen's group even two years after the publication of D2 were not advocating use of peptide ES1 for diagnosis of *M. tuberculosis* infection in humans is a clear indication that methods as now claimed were not obvious at the priority date. Indeed, if anything, D7 points away from use of any ESAT-6 peptide for detecting T cells in, or from, humans activated by *M-tuberculosis* infection.

Hence, it is believed that all the claims are linked by a novel and inventive unifying concept as recognized for the equivalent claims of the Parent International Application in the relevant IPER.

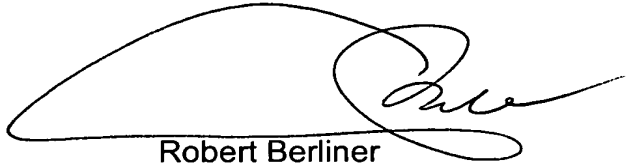
Species election in relation to SEQ. ID NOs

The Examiner's suggested need for a species election in relation to a SEQ ID No. from the SEQ ID Nos. recited in the claims other than SEQ ID No 1, is believed to not be appropriate. The peptide of SEQ ID No 1 (peptide ES1) is indicated by the specification to be the key peptide for determining *M. tuberculosis* infection in humans and as indicated above claims 27 and 28 direct use of that peptide alone or as part of a peptide panel with additional peptides selected from the peptides represented by any of SEQ ID nos. 2 to 11. The peptides of SEQ ID Nos: 2 to 11 are not alternatives for use in substitution for the peptide of SEQ ID No 1. Having regard to table 1 on page 26 of the specification, some preference might be given to peptide ES2 (SEQ ID No 6) for inclusion in any peptide panel with peptide ES1. However, as indicated above, claims 30 and 75 specify a preferred peptide panel consisting of all of SEQ ID NOs: 1 to 8.

Response in conformance with 37 C.F.R. § 1.143

Conforming with the requirements of Response in conformance with 37 C.F.R. § 1.143, applicant provisionally elects the claims of Group I (claims 27 - 43) for further prosecution in this application in the event the Examiner adheres to the requirement for restriction. In addition, applicant provisionally elects SEQ ID NO:6 as the second SEQ ID NO in the event no generic claim is finally held to be allowable

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Robert Berliner', is written over a large, loopy horizontal line that serves as a decorative underline.

Robert Berliner
Registration No. 20,121

Tel.: (213) 892-9200
Fax: (213) 680-4518